

## Effect of Using *Lactobacillus Brevis* IBRC-M10790 and Baker's Yeast on Phytate Degradation of Sourdough and Barbari Bread

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**ABSTRACT:** The possible use of *Lactobacillus brevis* IBRC-M10790 with degrading phytase activity was investigated in sourdough and Barbari bread –using 20 and 30% of sourdough in the formulation- in order to enhance the nutritional bioavailability, and the efficiency of the bacteria in decreasing phytate via using in sourdough was compared to yeast. Results showed that *L. brevis* IBRC-M10790 has better efficiency in phytate reduction than yeast alone or the combination of yeast and bacteria, and can be effectively used for improving the nutritional properties of Barbari bread. Use of 30% sourdough in bread formulation gave the highest percentage of phytate reduction in breads. It seems that not only the percentage of sourdough (the amount of used yeast), but also fermentation time is important in reducing the level of phytate in Barbari bread. Statistical analysis revealed that pH is not the major factor for phytate reduction in Barbari breads with different percentages of sourdough.

**Keywords:** Barbari Bread, *Lactobacillus brevis*, Phytate Reduction, Sourdough, Yeast.

### Introduction

Phytic acid (myoinositol 1, 2, 3, 4, 5, 6-hexakisdi-hydrogen phosphate, phytate or IP6), is the major storage form of phosphorous, comprising 1–5% by weight in cereals, legumes, oil seeds and nuts (Gupta *et al.*, 2015; García-Estépa *et al.*, 1999). It has the ability to chelate multivalent metal ions, especially zinc, calcium and iron, as well as resulting in poor bioavailability of minerals (García-Estépa *et al.*, 1999; Gupta

*et al.*, 2015). Degradation of phytate occurs via the enzyme phytase (EC 3.1.3.8). This enzyme can be derived from different sources, such as animals, plants and microorganisms (De Angelis *et al.*, 2003; Leenhardt *et al.*, 2005; Matsuo *et al.*, 2012). Considering fermented bakery products, phytase activity has been shown in different strains of commercial baker's yeast (Türk *et al.*, 2000; Lambrechts *et al.*, 1992), however, it seems not to be sufficient for the degradation of phytate. Thus, the addition of phytase or micro-organisms producing

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phytase was proposed by various researchers (Lopez *et al.*, 2000; Sandberg and Andlid, 2002; De Angelis *et al.*, 2003; Reale *et al.*, 2007; Palacios *et al.*, 2008; Didar and Haddad Khodaparast, 2011). Nevertheless, different reports have revealed that phytase activity in lactic acid bacteria seems to be strain-specific (Reale *et al.*, 2007; Lopez *et al.*, 2000).

Bread made from wheat is a staple food in many countries, especially in Iran. Its consumption is 140-160 kg per year (more than twice of what is consumed in Europe) (Abtahi *et al.*, 2014). Since bread is consumed at almost every meal, it is of utmost importance in the case of nutrition bioavailability. Thus, reducing phytate should be given great attention. Among four types of bread mainly consumed in Iran, Barbari is the most popular one, and its taste becomes unique when produced by sourdough (Pourafshar *et al.*, 2015).

The use of sourdough in bread processing has a long tradition and plays an important role in the quality and nutritional value of different breads (Diowksz and Kordialik-Bogacka, 2017), especially in the case of IP6 reduction (Najafi *et al.*, 2012; Poutanen *et al.*, 2009; Gobetti *et al.*, 2005; Gobetti *et al.*, 2014). In addition, using lactic acid bacteria (LAB) in sourdough has received extensive attention, due to their impact on different characteristics of the dough and bread. Phytase is one of the metabolites produced by some species of LAB (Reale *et al.*, 2007; De Angelis *et al.*, 2003; Didar and Haddad Khodaparast, 2011). According to different published reports, there is still a challenge between the role of yeast and LAB, regarding IP6 degradation. Leenhardt *et al.* (2005) illustrated the effect of dough acidification in the phytate breakdown either by LAB or lactic acid. However, Reale *et al.* (2007) have reported that LAB do not have a

direct effect on phytate degradation, and they carry out their function by lowering pH, in order to provide favorable conditions for endogenous cereal phytase. On the other hand, Palacios *et al.* (2008) stated that in the presence of certain bifidobacterial strains, reducing pH is not the main factor for hydrolyzing phytase.

Several studies have been carried out on the purification and characterization of phytase from different LAB or evaluating its activity in various foods (Sumengen *et al.*, 2013; Zamudio *et al.*, 2001; Reale *et al.*, 2007; De Angelis *et al.*, 2003; Lopez *et al.*, 2000; Tang *et al.*, 2010), however, this study aims to use *Lactobacillus brevis* IBRC-M10790 in sourdough, to improve the nutritional value of bread by producing phytase, and comparing its effect with yeast on phytate degradation, as well as evaluating the effect of pH for providing an optimum condition. To the best of our knowledge, this novel strain of LAB isolated from Tarhana (Tafvizi and Tajabadi Ebrahimi, 2015), has not been used in any food matrix, for verifying its phytase production.

## Materials and Methods

### - Materials

The flour utilized in this study for measuring IP6 quantity, producing sourdough and Barbari bread was commercial-type wheat flour (Tehran Bakhtar Co., Tehran, Iran) with an extraction rate of 79%. Flour characteristics are listed in Table 1. Salt was obtained from the local market. A typical fresh baker's yeast (*Saccharomyces cerevisiae*) was purchased from Iran Mayeh Co., Tabriz, Iran. *L. brevis* IBRC-M10790, was used as a sourdough starter, obtained from Tak Gen Zist Co. (Tehran, Iran).

**Table 1.** Chemical and rheological characteristics of the flour

Characteristics	
<i>Chemical</i>	
Moisture (%)	14.18
Wet Gluten (%)	30.2
Protein (% of dry weight basis)	12.5
Ash (% of dry weight basis)	0.66
<i>Rheological</i>	
Farinograph water absorption (%)	54.1
Dough development time (min)	2.06
Dough stability (min)	18.03
Falling Numbers	364

Mixing properties of the flour were determined using a Farinograph according to AACC method (54.21 on 50.0 g of the flour) (AACC, 2000).

Values represent the average of three replications.

#### - Methods

##### - Starter Preparation

*L. brevis* IBRC-M10790 was received in a lyophilized form, and then grown in a sterile industrial broth culture for 12 h in a 37°C incubator. Prior to this step, bacteria were incubated for 5, 12, 24 and 48 h, and results showed that 12 h is the best time for incubation, as some derivatives were consumed by the bacteria after this time. After incubation, all broth medium within the cells were utilized in the preparation of sourdough with the cell density of  $1.0 \times 10^9$  CFU/ml.

##### - Sourdough preparation

Sourdoughs were prepared in a spiral mixer (Diosna Co., model SP12-SP160, Germany), by mixing 500 g wheat flour with 310 g tap water, 10 g salt, 20 g yeast (in the trials which yeast was included) and the inoculum of the sourdough starter ( $1.0 \times 10^9$  CFU/ml) for 2 min in slow speed and 6 min fast speed. For the preparation of sourdough starter, *L. brevis* IBRC-M10790 was incubated for 12 h in MRS broth (De Man *et al.*, 1960) at 37°C in an aerobic condition. Prepared sourdoughs were thereafter put in the bowl, covered with a plastic cover and held for 24 h in room temperature.

##### - Determination of pH

pH measurements were carried out according to AACC method (02-52) in triplicate order.

##### - Bread production

For the preparation of Barbari bread, 600 g of flour, 10.8 g of salt, 24 g of fresh yeast, and 420 ml of water were utilized (control bread). For evaluating the effect of sourdough on phytate reduction in bread, Barbari samples were prepared using two different amounts of sourdough: 20 g/100 g of dough (formulation 1), and 30 g/100 g of dough (formulation 2). All ingredients were mixed and kneaded in a spiral mixer (Diosna, model SP12-SP160, Germany) for 2 min slow speed and 6 min fast speed. The prepared dough was divided into 500 g pieces and put into proofer (38°C, RH=85%) for 20 min. Thereafter, semi-proofed dough pieces were brought out from the proofer, formed to the flattened shape by hands, punched by fingers, and continued proofing for 6 min. Baking was carried out in a Miwe rack oven (Germany) in 250°C for 11 min. Breads were cooled after 30 min and packed in plastic bags for further analysis.

##### - Sample preparation

Phytate was extracted from the samples

based on the method described by Dost and Tokul (2006). The extracts were centrifuged at 12000×g for 15 min (Micro 200R, Hettich Co., UK), while the supernatants were filtered through a 0.22 µm filter (Sartorius No. 17593Q, Germany).

#### - Phytate analysis by HPLC

HPLC analysis was conducted following the chromatographic method described by Lehrfeld (1994) with some modifications. Briefly, a PLATINblue UHPLC-System (Knauer, Germany) was used, equipped with an L-7100 pump. Samples (10 µl) were loaded onto a Hamilton anion exchange PRP-X 100 column (PRP-X100 5 µm 4.1 x 150 mm, Knauer, Germany) with an automatic injection system, a Photodiode array detector (PDA) set at 460 nm, operated using EZchrom software. Elution was performed at a flow rate of 1 ml/min, at 45°C using an isocratic mobile phase consisting of water phase A: 0.5 ml formic acid, 8 ml of 0.04M Tetrabutylammonium hydroxide solution (TBAOH), and adjusting the pH to 4.1 with 5 M sulphuric acid; Phase B: acetonitrile. All chemicals were of chromatographic and analytical grade, obtained from Merk Millipore Co., Germany. Commercially available (Sigma-Aldrich) D-myo-inositol 1, 2, 3, 4, 5, 6-Hexakisphosphate, and dodecasodium salt (Cat. No. 407125, CAS No. 14306-25-3) were used as standards. Samples were run in duplicate.

#### - Statistical analysis

All the statistical analyses were carried out using the SPSS software, version 23 (IBM, USA). Values are given as the means ± standard error. Significant differences among means were determined by one-way ANOVA. The level of statistical significance was set at  $P < 0.05$ .

### Results and Discussion

Phytate content of the flour was obtained

as  $300.494 \pm 0.01$  mg/ 100 g of dry weight basis. After 24 h fermentation in the control sourdough (prepared by yeast), it decreased to  $121.272 \pm 0.11$  mg/ 100 g of dry weight basis (60% reduction) ( $P < 0.05$ ). García-Estépa *et al.* (1999). reported almost the same amount of phytate for commercial white wheat flour, and 50% reduction of phytic acid for commercial breads. By using both yeast and *L. brevis* in the formulation of sourdough, phytate content was reduced to  $95.927 \pm 0.01$  mg/ 100 g of dry weight basis ( $P < 0.05$ ), and when using *L. brevis* in sourdough without any added yeast, the amount of phytate was obtained as  $48.125 \pm 0.11$  mg/ 100 g of dry weight basis (68 and 84% loss of phytate, respectively) ( $P < 0.05$ ).

In control Barbari bread (without any added sourdough), phytate content was obtained as  $217.587 \pm 0.12$  mg/100 g of dry weight basis (28% reduction) ( $P < 0.05$ ). This loss may be the result of yeast activation or intrinsic phytases in flour in optimum condition of fermentation, as well as high temperature during baking (García-Estépa *et al.*, 1999). The use of sourdoughs in breads, either 20 or 30% of the formulation, resulted in further reduction of phytate content (Table 2). In other words, phytate reduction was significantly higher in Barbari breads with added sourdough ( $P < 0.05$ ). This might be attributed to the activity of both yeast and bacteria in sourdough and also their extra time for activity during the proofing of breads. Other reports also confirmed the effect of fermentation and sourdough in phytate reduction (Arendt *et al.*, 2007, Najafi *et al.*, 2012, Leenhardt *et al.*, 2005, Kasprovicz-Potocka *et al.*, 2017, Mohammed *et al.*, 2017) as well as the time dependency of phytase degradation (Palacios *et al.*, 2008, Türk and Sandberg, 1992, Mohammed *et al.*, 2017). All breads including 20 or 30% sourdough in the formulation (without *L. brevis*) showed approximately 96% phytate

reduction. Phytate content was obtained as  $11.335 \pm 0.08$  and  $11.563 \pm 0.08$  mg/100 g of dry weight basis, respectively ( $P < 0.05$ ). Adding *L. brevis* to the sourdough, showed a little more, but significant reduction of phytate in all breads. Phytate content for such Barbari breads with 20 or 30% sourdough was  $10.656 \pm 0.14$  and  $10.269 \pm 0.07$  mg/100 g of dry weight basis, respectively (about 96.5% reduction) ( $P < 0.05$ ). All data are summarized in Table 2.

#### - pH and Phytase Activity

pH of the flour and control bread without any sourdough, was measured as 6.1 and 5.9, respectively (Table 2). Adding sourdough to the bread formulation reduced the pH significantly ( $P < 0.05$ ). The most reduction was observed in breads with both yeast and bacteria sourdough. Comparing sourdoughs with each other, pH in the sourdoughs including *L. brevis* was lower (pH=4.52) perhaps due to the metabolites they produced. Sourdoughs with both yeast and bacteria (pH=5.11) and sourdoughs with yeast without bacteria (pH=5.39) were the next. Leenhardt *et al.* (2005) reported that pH of the dough was the main determining

factor for phytate hydrolysis in whole bread, and by using sourdough in the formulation, this optimum condition can be achieved. Požrl *et al.* (2009) also confirmed the influence of pH on phytic acid reduction. They stated that samples of lower pH had lower phytic acid content (Simčić, 2009). Didar and Haddad Khodaparast (2011) reported that pH and phytic acid content of breads follow nearly the same pattern. On the contrary, Palacios *et al.* (2008) stated that, reducing pH did not play any major role in phytate reduction, mainly in the presence of different strains of bifidobacterial species.

Regarding phytate degradation in sourdoughs, it can be seen that all three kinds of sourdoughs had phytate reduction. This confirms the previous reports that both yeast and bacteria have phytase activity (Reale *et al.*, 2004). Gupta *et al.* (2015) stated that the properties of different phytases (such as optimum pH, temperature, etc.) obtained from various sources exhibited differently. They reported the optimum temperature and pH of phytases in the range of 25 to 80°C, and 4.5 to 6, respectively. Regarding this, all the sourdoughs in this

**Table 2.** Phytate content, its residue and reduction percentage, and pH changes in flour, sourdoughs after 24 hours' fermentation, and Barbari bread

Samples	Phytate (mg/100g, dry weight basis)	Residue (%)	Reduction (%)	pH
Flour	$300.494 \pm 0.01$ a	$99.997 \pm 0.003$ a	$0.003 \pm 0.003$ i	$6.10 \pm 0.005$ a
C0	$217.587 \pm 0.12$ b	$72.410 \pm 0.04$ b	$27.59 \pm 0.04$ h	$5.90 \pm 0.010$ b
C20	$11.335 \pm 0.08$ g	$3.772 \pm 0.03$ f	$96.23 \pm 0.02$ c	$5.81 \pm 0.005$ c
C30	$11.563 \pm 0.08$ f	$3.848 \pm 0.03$ g	$96.15 \pm 0.02$ d	$5.78 \pm 0.000$ d
SDY	$121.272 \pm 0.11$ c	$40.356 \pm 0.07$ c	$59.64 \pm 0.07$ g	$5.39 \pm 0.005$ g
SDB	$48.125 \pm 0.11$ e	$16.015 \pm 0.03$ e	$83.99 \pm 0.03$ e	$4.52 \pm 0.030$ i
SDYB	$95.927 \pm 0.01$ d	$31.922 \pm 0.01$ d	$68.08 \pm 0.01$ f	$5.11 \pm 0.010$ h
TD4Y20	$10.656 \pm 0.14$ h	$3.546 \pm 0.05$ h	$96.45 \pm 0.05$ b	$5.60 \pm 0.010$ f
TD4Y30	$10.269 \pm 0.07$ i	$3.417 \pm 0.02$ i	$96.58 \pm 0.02$ a	$5.66 \pm 0.005$ e

TD4Y20: Barbari breads with 20% sourdough including yeast and *L. brevis*, TD4Y30: Barbari breads with 30% sourdough including yeast and *L. brevis*, C0: Barbari bread without sourdough, C20: Barbari bread with 20% sourdough including yeast, C30: Barbari bread with 30% sourdough including yeast, SDB: sourdough with *L. brevis* without yeast, SDY: sourdough with yeast without *L. brevis*. SDYB: sourdough with yeast and *L. brevis*.

Mean and standard error of two determinations, expressed on a dry weight basis.

Different letters in each column indicate statistically significant differences ( $P < 0.05$ )

study had such an optimum condition during fermentation. Sourdoughs containing *L. brevis* and not yeast, had the highest phytate reduction and the lowest pH=4.52. This may be as a result of the activity of lactic and acetic acids that are from the most produced metabolites of *L. brevis* IBRC-M10790 (data are not shown), and are effective in decreasing the pH of the dough. Although Türk *et al.* (1996) stated that organic acids such as lactic and acetic acids do not have a significant effect on phytate reduction, in the viewpoint of pH. This result is in line with previous works carried out by different researchers which had reported that the highest phytate degradation in baking studies is around 4.5 (Türk *et al.*, 1996, Larsson and Sandberg, 1991). Microbial phytases have been reported to be active over the wider range of pH than plant phytases (Türk and Sandberg, 1992), however, lower pH may have favored the activity of endogenous phytase (Türk *et al.*, 1996). The optimum pH for plant seeds phytases have been reported between 4 and 5.6 (Gupta *et al.*, 2015). As the pH for all sourdoughs in this study was in this range, intrinsic phytase of the flour may have also participated in the degradation of phytate in all samples.

Comparison of the pH of Barbari breads containing yeast with those containing both yeast and bacteria, showed that the pH of latter breads was significantly lower ( $P < 0.05$ ). Results of previous works showed that depending on the starter culture used, the pH of the sourdough varies (Palacios *et al.*, 2008, Esteve *et al.*, 1994). Esteve *et al.* (1994) also reported that the presence of yeast in the sourdough results in increased pH of breads. They also enumerated the percentage of sourdough as the most important factor affecting acidity. In our study, increasing the percentage of sourdough from 20 to 30 in breads containing yeast and not bacteria, reduced the pH of the breads significantly ( $P < 0.05$ ),

although phytate reduction did not increase. Nevertheless, using 30% sourdough containing *L. brevis* and yeast in breads significantly decreased the pH and also reduced the phytate of the bread ( $P < 0.05$ ). By achieving such a result, it can be concluded that in agreement with Palacios *et al.* (2008), pH may be helpful in speeding up the reduction of phytate, but is not the major factor for phytate reduction in Barbari breads with different sourdough percentage.

### Conclusion

In most bakery products such as bread, fermentation time is approximately 2 h, which is not enough for sufficient hydrolysis of phytate. Using sourdough in bread production with or without LAB can lead to phytate degradation, as there would be more time available for the activity of both yeast and bacteria. It seems that not only the percentage of sourdough (the amount of used yeast), but also fermentation time is important in reducing phytate level in Barbari bread. Results of this study also revealed that *L. brevis* IBRC-M10790 has better efficiency in phytate reduction than yeast alone, as well as the combination of yeast and bacteria, and can be effectively utilized for enhancing the nutritional properties of Barbari breads. Statistical analysis did not show a strong correlation between pH and phytate reduction. Perhaps pH was the minor factor for phytate reduction.

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